The work to be presented in the thesis entitled “STUDIES ON ANTIBIOTIC ELUTING HYDROXYAPATITE COATED ORTHOPAEDIC IMPLANT” has been divided into five chapters.

Thesis begins with the introduction described in **Chapter-1.**

Presently major medical devices for different applications like, Orthopedics, ENT, Cardiovascular, Dental etc. are made up of metals viz. SS 316L, Co-Cr alloy, Ti6Al4V and Titanium. Major advantage for their penetration is its mechanical strength and proven biocompatibility. The aim of Orthopaedic implant is to maintain stability until fusion or fracture healing has occurred.

Bones, which usually are well protected from infection, can become infected by bacteria and fungi directly through open fractures, during bone surgery or from contaminated objects that pierce the bone [1]. Several procedures for treatment include removal of the infected device, long term systemic antibiotic therapy with all it side effect and sometimes requires removal and further implantation through surgery [3]. This is a serious concern for the patients as well as surgeons. Conventional therapy with systemic antibiotics (orally or parentally) often becomes unsuccessful due to poor antimicrobial distribution at the site of infection and poor blood circulation to skeletal tissue [4]. Systemic antibiotic therapy alone does not eradicate bacteria because of poor penetration into bone [6]. Staphylococcus aureus is the bacteria most commonly responsible for bone and tissue related infection [8]. Thus, there is a need to achieve prolonged drug delivery that can persist at least over a month, to prevent post operative orthopedic infections.

Local drug delivery is a well known concept in the medical industry (devices include drug coated coronary stent, delivery catheters, drug loaded beads, drug loaded cements for orthopedic applications etc.) to provide drug directly to the target area without exceeding higher drug levels in different parts of body. Drug impregnated PMMA bone cements/Beads have been commercially made available for clinical use. However, PMMA is not biodegradable and clinical failures can occur due to difficulty of bone regeneration & despite their antibiotic release, these beads act as a biomaterial surface to which bacteria preferentially adhere, grow and potentially develop antibiotic resistance [11]. Several researchers have focused on use of drug

impregnated ceramic materials such as tri-calcium phosphate or hydroxyapatite, since their chemical composition is similar to bone [13]. Recently, peptide based implants, the so called RGD (arginine, glycine, aspartic acid)- peptide, have been reported to stimulate the adhesion of osteoblasts and, therefore, to improve the osteointegration of RGD-coated implants[14]. The incorporated antibiotics showed a continuous release for a period of about 96 h with an initial peak of release in the first 6 h [16]. That means the antimicrobial effect exists only for a few days, such high doses and/or lengthy treatment (orally or parentally in high dose) with antibiotics often lead to adverse effects like ear and kidney damage [18]. Controlled release of antimicrobial drugs from the implanted objects thus represents an alternative to conventional systemic therapy [21].

Second most frequent drawbacks in Orthopaedic surgery are loosening of implants, corrosion of metal, leaching of ions and foreign body adverse reactions. Generally Implants have to be tight with surrounding bone tissue. Loosening of the implants results in a loss of stability which might lead to non-union or loss of reduction [25]. The fixation of orthopaedic implant depends on several factors like the quality of the bone, the design and size of the implant, chemical stability, mechanical behavior, biocompatibility in body fluids and tissues are the basic requirements for successful application of implanted materials in bone fractures and replacements [29].

Various Bio-ceramics like-Alumina, Zirconia, Titania, Bioglass, Silica, Calcium phosphate group etc. have been matter of interest for scientists for their higher biocompatibility over metals[30-31]. Due to their brittle nature and low load bearing capacity, they are not widely popular to be used as prosthesis and alternatives for metallic implants [32]. Hydroxyapatite (HAp) is a class of calcium phosphate containing water in the chemical composition as $Ca_{10}(PO_4)_6(OH)_2$ with a Ca/P molar ratio is 1.67. HAp is the least degradable form among various calcium phosphates, insoluble in neutral and alkaline solutions [33]. Hydroxyapatite has a very similar chemical composition like the inorganic part of human hard tissue, such as bone and teeth [34].

In brief, Dual coating was developed on metallic implant: (1) Hydroxyapatite coating on metallic implant for improving biocompatibility, bond strength with bone tissue

and (2) Antibiotic eluting polymeric coating that was done only one side of the Hydroxyapatite coating (Figure-1).

In present research work, coating of antibiotic drug on metallic implants using biodegradable polymer Poly (D, L- Lactide) as a carrier in multi layers control release has been investigated (Chapter 3). The main objective of the present investigation is to develop and examine drug loaded biodegradable polymer films that can bind to the orthopedic implants and prevent bacterial infection through controlled release of the drug. The release kinetics should be such that the drug is delivered at least for a period of one month so that no complications arise during the osteointegration period. Coating should be non-cytotoxic, with minimum platelet adhesion and non-pyrogenic.

To develop hydroxyapatite coating various coating techniques like Electrophoretic deposition, Electrochemical deposition, Sol-gel, plasma Spray and brush coating were employed (chapter 4). The Hydroxyapatite coating should be biocompatible, non-pyrogenic, having sufficient adhesion to implant surface and Ca/P ratio nearest to 1.67.

The final product “Antibiotic Eluting Hydroxyapatite coated Orthopaedic Implant” was prepared (chapter 5). Different techniques i.e. Scanning electron microscopy, Drug release, Antimicrobial activity test, Bacterial Endotoxin test were used for evaluation of the quality of implant.

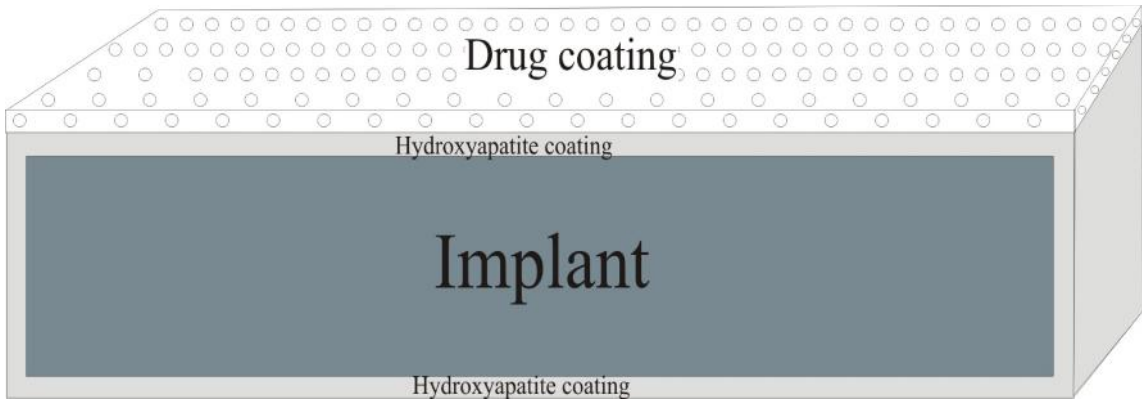

Figure 1. Schematic Diagram of Antibiotic Drug Eluting Hydroxyapatite coated Implant.

Chapter-2 describes various kind of materials used during the experimental work and is divided according to their use.

Gentamicin (Triomphe Fine Chemistry Company Ltd., China) and Cefazoline (Ranbaxy Laboroatory, India) were used without further purification. Polymer; Poly (D, L- lactide) (Bio Invigor Corporation; Taiwan) having inherent viscosity 0.594 dl/g was used as a carrier for the drug.

The solvents viz. Acetone GR, Acetone HPLC, HPLC water (Merck, India), 2-Propanol (Merck, India) and Ethanol AR (Changshu Yangyuan Chemical, China) used during the investigation.

The strips of the dimension 1.5 cm x 3.0 cm x 0.25 cm SS 316L (ASTM-F 138), cortical screws (Biomed Corporation, India) and 1.0 cm x 1.0 cm x 0.5 mm Titanium strips (Matrix Meditech Pvt. Ltd., India) were used as substrate for coating.

Triethyl Phosphite (Labort Fine Chem, India, Calcium Nitrate tetrahydrate (Merck, India), Ammonium dihydrogen orthophosphate (Merck, India), Hydroxyapatite (Clarion Pharmaceutical, India) were used for Hydroxyapatite coating.

Staphylococci aureus 25923 ATCC and E-coli 25923 ATCC (Quantum Biotech, Mumbai) were used for the antimicrobial activity of Gentamicin eluting Orthopaedic system by a Kirby-Bauer technique.

Chapter-3 gives the detailed studies on Gentamicin eluting orthopaedic implant

Following method was for the preparation of Samples:

Biodegradable polymer; Poly (D, L- Lactide) was dissolved in HPLC grade acetone (solution A) and fixed quantity of Gentamicin was dissolved in water (solution B). Coating solution was formulated by mixing of both solution A and solution B at the proportion of 20% Gentamicin and 80% Poly (D, L- Lactide). Strips were stored in amber colored glass vials after washing with de-ionized water followed by acetone and followed by vacuum drying for solvent evaporation. Before coating, the strips and cortical screws were weighed using analytical balance (Citizen Model CX-220) having 0.01 mg accuracy. Base layer of Strips and Cortical screws were coated by aerosol spray technique to achieve a loading of 30 $\mu g/cm^2$ Gentamicin. Further only polymeric top layer of Poly (D, L- Lactide) was coated for protection against moisture on base layer and to prevent premature drug release. Coated materials were followed by vacuum drying for solvent evaporation. All coated samples were sterilized by Ethylene oxide.

Various tests were conducted to evaluate the efficacy of Gentamicin eluting orthopaedic implant,

- Drug Loading test
- Drug Purity test report
- Scanning Electron Microscopy
- Coating thickness measurement
- Drug release test
- Antimicrobial Activity test
- In-vitro cytotoxicity test
- Platelet adhesion
- Rabbit Pyrogen test
- Residual ETO
- Residual solvent
- Sterility test report
- Drug- polymer Interaction

Chapter-4 Gives various coating techniques for Hydroxyapatite coating on orthopaedic implant and different properties exhibited as well as their characterization. Coating techniques for Hydroxyapatite coating used were

- Electrophoretic Deposition
- Electrochemical deposition
- Sol-gel technique
- Plasma Spray coating
- Brush Coating

The Adhesion test was conducted for all the techniques used for sample preparation of Hydroxyapatite coated titanium implant. Below mentioned studies were performed on the samples of Hydroxyapatite coated titanium implant that was made by Brush coating technique;

- Adhesion test
- Scanning Electron Microscopy
- EDAX

- Coating Thickness measurement
- LAL test
- Dissolution / Degradation in Phosphate buffer solution
- X Ray Diffraction
- FTIR
- Optical image

Chapter-5 Discusses the Cefazolin eluting Hydroxyapatite coated orthopaedic implant and the results obtained by performing different tests on the samples. The Samples were prepared using Hydroxyapatite coating on Titanium plate by Brush coating technique mentioned in chapter-4. Then further Cefazolin / PDLLA matrix was coated on only one side of Hydroxyapatite coating. Following tests were conducted for the elution characteristics of prepared samples.

- Drug release test
- Antimicrobial Activity test
- Degradation behavior in PBS
- Bacterial Endotoxin test
- Scanning Electron Microscopy
- Optical Image

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